

Antioxidant Potential of Carica Papaya Peel and Seed

Yee Kwang Ang¹, Winne CM Sia², Hock Eng Khoo³, Hip Seng Yim⁴

^{1,2,4}Department of Food Science and Nutrition, Faculty of Applied Sciences, UCSI University, No. 1 Jalan Menara Gading, UCSI Heights, 56000 Kuala Lumpur, Malaysia.

³Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

¹kayvi21@gmail.com; ²siacm@ucsiuniversity.edu.my; ³hockeng_khoo@yahoo.com; ⁴hsyim@ucsiuniversity.edu.my

Abstract

The present study aimed to evaluate the antioxidative potential of selected Carica papaya peel and seed by determining their total phenolic content (TPC) and antioxidant activities. TPC was determined by Folin-Ciocalteu method, while antioxidant activities were evaluated using DPPH radical scavenging ability, ferric reducing/antioxidant power (FRAP), and ABTS radical cation inhibition activity. Papaya peel was extracted using 90% acetone (v/v) for 60 min showed the highest TPC of 15.18 µg GAE/mL extract. Antioxidant activities measured by DPPH, FRAP and ABTS assays were 37.34%, 19.70 µg TE/mL extract, and 28.30%, respectively. On the other hand, extraction using deionised water for 120 min for papaya seed had the highest TPC of 6.75 µg GAE/mL extract, while the antioxidant activities obtained for DPPH, FRAP and ABTS assays were 57.30%, 16.05 µg TE/mL of extract, and 11.19%, respectively. Papaya peel and seed extracts demonstrated potent antioxidant activity to a certain extent and could be of nutraceutical importance for food industry application.

Keywords

Antioxidant Activity; Carica Papaya; Peel; Seed

Introduction

In recent years, the role and beneficial effects of many phytonutrients from plant sources such as fruits and vegetables had attracted much attention from food scientists as well as the public. These phytochemicals are natural antioxidant which frequently promoted due to the concerns regarding toxicity of the synthetic ones. Apart from free radical scavenging activity, antioxidants found from most of the plants possess antibacterial, antiviral, antimetastasis activity, antiulcer activity, antimutagenic, antiallergic, anticarcinogenic effects, and anticarcinogenicity [1].

Increasing numbers of studies on residual sources in replacing synthetic antioxidants with the natural ones

are due to the augmented recycling interest of the agro-food industry. These are playing an important role in improving the complete utilization of the residual sources. The wastes or by-products from food processing such as seed and peel of some fruits contain higher source of potential antioxidant activities than that the edible portion, for example, pomegranate peel and grape seed had been shown to possess higher antioxidant activity than their pulp [2]. However, there are limited by-product derived antioxidants that were successfully developed except grape seed and olive waste extract in the European food processing industry [3].

Papaya, as a tropical fruit, was traditionally used as therapeutic remedy due to its medicinal properties. The fruit is rich in phytochemical, especially carotenoids and polyphenols [4]. Papaya is also known to be a thirst quencher by people living in tropical countries. Besides its juicy pulp, the peel and seeds of papaya are valuable too. Papaya seeds were known to give several health benefits [5, 6]. In some of the poor countries in tropical region, papaya peel is used for cooking as one of the dishes. Although papaya peel and seeds have various uses, the phytochemicals especially phenolic compounds in these parts of papaya have antioxidative properties. Therefore, this study aimed to evaluate the antioxidative potential of *Carica papaya* var. Sekaki peel and seed extracts by determining the total phenolic content (TPC) and antioxidant capacities using various biochemical assays.

Materials and Methods

A. Sample Preparation

Fully matured papaya (5 kg) was purchased from selected night market in Kuala Lumpur, Malaysia. The

papayas were washed under running tap water and the seed and peel of papaya were separated from the pulp. Papaya peels were cut into small pieces of about 1 cm² before drying in convection oven (Memmert, Germany) at 45°C. Papaya samples were milled at Forest Research Institute of Malaysia (FRIM) using the miller (QUADRO COMIL, Quadro Engineering, Canada) at 3873 rpm which yielded particle size of 813 micron. All samples were vacuum-packed using vacuum packager (DZQ400/500, China) until further analysis.

B. Sample Extraction

The sample was pre-tested under various extraction parameters included different extraction media, solvent concentration and extraction time. Briefly, 10 g of sample (dried papaya peel and seed) was extracted with 100 mL of selected extraction media (acetone and deionised water, respectively). The sample was filtered through Whatman No.1 filter paper and was re-extracted under same conditions. The filtrates were combined and collected in the amber reagent bottle.

The pooled extracts were centrifuged using a centrifuge (UNIVERSAL 320R, Hettich Zentrifuge, Germany) at 4500 rpm for 10 min. Supernatants were collected and the combined extracts were concentrated under vacuum (Rotavapour R-200, BUCHI, Switzerland) at 45°C. Analyses of TPC and antioxidant capacities were performed for papaya peel and seed extracts based on optimised extraction conditions (90% acetone and 60 min extraction for papaya peel; deionised water with 120 min extraction for papaya seed). These optimized conditions were determined using various solvent systems and extraction times as reported elsewhere [7].

C. Total Phenolic Content

The total phenolic content (TPC) was assayed colorimetrically using the Folin-Ciocalteu's reagent (FCR) method, as describe by Dubost et al. [8] and Ferreira et al. [9]. Briefly, 1 mL of sample extract was mixed with 4 mL of FCR reagent (prepared previously using 10-fold dilution with deionised). After 3 min, the mixture was added with 5 mL of 7.5% sodium carbonate solution. The mixture was shaken vigorously using a vortex (VORTEX V-1, BPECO, Germany) and incubated at room temperature in the dark for 30 min. The deionised water was used as blank. Finally, the absorbance was read at 765 nm using a PRIM visible spectrophotometer (Secomam,

France). TPC was expressed as gallic acid equivalents (GAE) per mL of extract.

D. DPPH Radical Scavenging Ability

DPPH radical scavenging ability was done according to the method of Tsai et al. [10]. Briefly, DPPH reaction mixture was shaken vigorously using vortex and incubated for 30 min at room temperature in the dark. The negative control was prepared as above without any extract, and ethanol was used as blank. The changes in absorbance of the samples were measured at 517 nm using a spectrophotometer. The DPPH scavenging ability of the sample was calculated using the following equation:

DPPH radical scavenging ability (%)

$$= \left[1 - \frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \right] \times 100$$

The percentage of scavenged DPPH was plotted against the natural logarithm (Ln) of a series of sample concentration to calculate the amount of free radical scavenging compound required in reducing the initial DPPH concentration by 50% graphically (EC₅₀ was expressed in µg/mL).

E. Ferric Reducing/Antioxidant Power Assay

The ferric reducing/antioxidant power (FRAP) was estimated based on a method described by Biglari et al. [11] with slight modification. The working FRAP reagent was prepared by mixing 2.5 mL of 10 mM TPTZ solution in 40 mM hydrochloric acid with 2.5 mL of 20 mM FeCl₃·6H₂O and 25 mL of 0.3M acetate buffer at pH 3.6. The freshly prepared FRAP reagent was incubated in water bath (WB/OB 7-45, Germany) at 37°C prior to use. Briefly, 3 mL of freshly prepared FRAP working reagent was added into a test tube containing 100 µL of sample and followed by the addition of 300 µL of deionised water. The absorbance was read against reagent blank after 4 min. The FRAP value was expressed as Trolox equivalent (TE) per mL of extract.

F. ABTS Radical Cation Inhibition

The radical inhibition activity of the samples against ABTS radical cation was carried out according to the method described by Cheung and Lo [12]. ABTS radical cation was produced by reacting 5 mL of 7 mM ABTS with 88 µL of 140 mM potassium persulfate (K₂S₂O₈) and allowing the mixture to stand in the dark at room temperature for 16 h. The ABTS solution was

diluted with 95% ethanol in order to get an absorbance of 0.70 ± 0.05 at 734 nm. A 1 mL of ABTS solution was mixed with 10 μ L of sample solution after 6 min incubation before the monitored using a spectrophotometer at 734 nm against the ethanol blank.

The inhibition percentage of the sample against the ABTS radical cation was calculated using the following equation; the EC_{50} was also calculated and expressed as mg/mL.

$$\% \text{ Inhibition} = \left[1 - \frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \right] \times 100$$

G. Statistical Analysis

All experiments were carried out in triplicates and Statistical Package for Social Sciences (SPSS, version 18.0) was used for data analysis. The data collected were expressed as mean \pm standard deviation. Whenever applicable, t-test and one-way analysis of variance (ANOVA) with post-hoc Tukey's test were used to determine the significant differences among the variables. The level of significant difference was set at $p < 0.05$.

Results and Discussion

A. Determination of Total Phenolic Content

As shown in Figure I, the TPC of papaya peel increased with an increased sample concentration, where values range from the lowest 1.42 ± 0.06 μ g GAE/mL extract to the highest 15.18 ± 0.07 μ g GAE/mL extract. On the other hand, the TPC values of papaya seed were range from the lowest 0.60 ± 0.05 μ g GAE/mL extract to the highest 6.75 ± 0.08 μ g GAE/mL extract. Generally, the papaya peel extract has higher TPC as compared to papaya seed extract. It is likely that the high level of TPC in the peel is due to the protective activities of antioxidants, which are essential to protect the fruit and its seed from harmful environmental factors. The result was in agreement with the study of Ismail et al. [13] which reported that the total phenolic content in the fruit skin was higher than its seed of cantaloupe extracts.

B. Evaluation of DPPH Scavenging Activity

The DPPH radical scavenging activity of papaya samples were compared with those of known natural and synthetic antioxidants (α -tocopherol, ascorbic acid (AA), and BHA). The scavenging effect of DPPH radicals assay showed concentration-dependent activity. As shown in Figure II, α -tocopherol, BHA and

AA showed the highest DPPH radical scavenging activity, while the scavenging activity of both papaya peel and seed extracts were significantly lower compared to synthetic antioxidants. Result also showed that α -tocopherol exhibited highest free radicals scavenging activity at all concentrations studied, followed by BHA, AA, papaya seed and peel extracts.

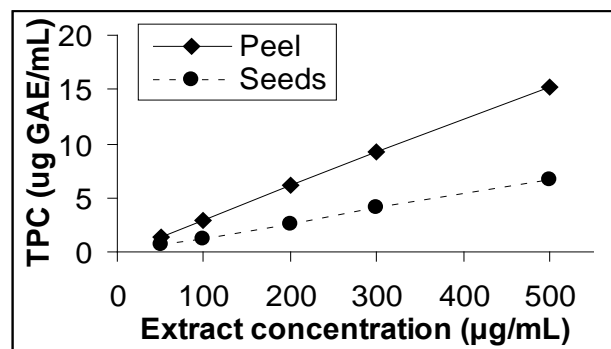


FIGURE 1 TOTAL PHENOLIC CONTENT OF PAPAYA PEEL AND SEED EXTRACTS. RESULTS ARE PRESENTED AS MEAN \pm STANDARD DEVIATION (N=3)

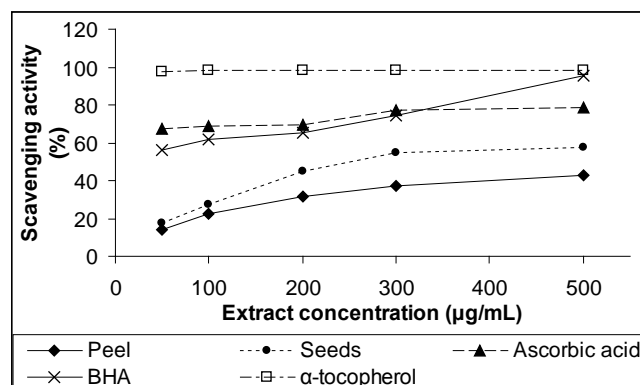


FIGURE 2 DPPH RADICAL SCAVENGING ABILITY (%) OF PAPAYA PEEL AND SEED EXTRACTS. RESULTS ARE PRESENTED AS MEAN \pm STANDARD DEVIATION (N=3)

TABLE I EC_{50} OF PAPAYA PEEL AND SEED EXTRACTS ASSESSED USING DPPH RADICAL AND ABTS RADICAL CATION SCAVENGING ASSAYS

Sample	DPPH Assay	ABTS Assay
	EC_{50} (μ g/mL)	EC_{50} (mg/mL)
Papaya peel	810.43	5.47
Papaya seed	285.77	9235.84
Ascorbic acid	2.36	0.081
BHA	44.80	< 0.001
α -tocopherol	< 1	< 0.001

EC_{50} : The effective concentration at which the antioxidant activity using DPPH/ABTS free radical was scavenged by 50%.

The DPPH radical scavenging activity of papaya peel increased with the increased extract concentration, which ranged from the lowest $14.23 \pm 0.88\%$ to the highest $37.34 \pm 1.18\%$ at the sample concentration of $300 \mu\text{g/mL}$. On the other hand, the DPPH radical scavenging activity of papaya seed had the values ranged from the lowest $17.59 \pm 0.79\%$ to the highest $57.30 \pm 0.41\%$ at the sample concentration of $500 \mu\text{g/mL}$. As shown in Table I, DPPH radical scavenging activity was also expressed in terms of EC_{50} . Lower EC_{50} value indicates higher radical scavenging activity of a sample. The EC_{50} values were ranged as follows: α -tocopherol ($< 1 \mu\text{g/mL}$), followed by AA ($2.36 \mu\text{g/mL}$), BHA ($44.8 \mu\text{g/mL}$), papaya seed ($285.77 \mu\text{g/mL}$ extract) and papaya peel ($810.43 \mu\text{g/mL}$ extract).

As revealed by Ahmadi et al. [14], DPPH method measures the ability of antioxidants present in scavenging the hydrophilic free radicals. In line to this theory, papaya seed have better ability in scavenge hydrophilic free radicals as compared to papaya peel that might due to the presence of hydrophilic antioxidants since deionised water was used in the extraction. Furthermore, the high antioxidant activity could be due to the increased in hydroxyl groups or amino groups in antioxidant compounds found particularly in the papaya seed extract [15].

C. Evaluation of FRAP

As depicted in Figure III, α -tocopherol, BHA and AA showed the highest radical TE values, while the TE values of both papaya peel and seed were much lower compared to the natural and synthetic antioxidants. At all extract concentrations, BHA had the highest TE value. AA ranks second, where it showed the lowest value of $47.36 \pm 1.56 \mu\text{g TE/mL}$ to the highest value of $1460 \pm 20.9 \mu\text{g TE/mL}$; followed by α -tocopherol, papaya peel and seed extracts. At concentration of $200 \mu\text{g/mL}$, the TE values decreased significantly in the order of BHA ($1478 \pm 131.5 \mu\text{g TE/mL}$) > AA ($1269 \pm 40.8 \mu\text{g TE/mL}$) > α -tocopherol ($584.7 \pm 40.7 \mu\text{g TE/mL}$) > papaya peel ($9.48 \pm 0.49 \mu\text{g TE/mL}$) > papaya seed ($8.90 \pm 0.34 \mu\text{g TE/mL}$).

FRAP assay measures the tendency of an antioxidant which act as a reducing agent and contribute a single electron to the Fe^{3+} in a redox-linked colorimetric reaction based on the breaking of the free-radical chain in order to stabilize and terminate the radical chain reactions [16]. This will cause an increase in absorbance; however, it is limited to only hydrophilic antioxidants or water-soluble antioxidants [17].

Besides, the reducing power of papaya peel and seed extracts showed a concentration-dependent activity.

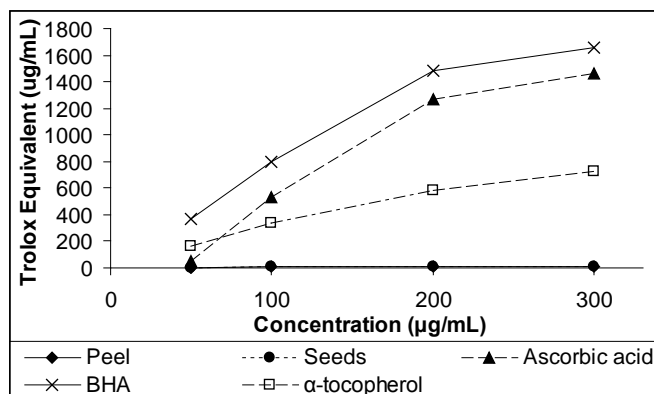


FIGURE 3 FRAP VALUE OF PAPAYA PEEL AND SEED EXTRACTS. RESULTS ARE PRESENTED AS MEAN \pm STANDARD DEVIATION (N=3).

D. Evaluation of ABTS Radical Cation Inhibition

The ability of the antioxidant compounds in sample extracts to inhibit the ABTS radical cation are measured using ABTS assay in comparison with natural and synthetic antioxidants (α -tocopherol, AA, and BHA). ABTS assay measures both the hydrophilic and lipophilic antioxidants since the reagent dissolve well in both aqueous (hydrophilic) and organic solvents (hydrophobic) and their chromophore or fluorescent species are in neutral or univalent-charged [18]. Besides, the scavenging effect on ABTS radicals assay showed concentration-dependent activity.

As shown in Figure IV, α -tocopherol, BHA and AA showed the highest ABTS radical cation inhibition activity, while the inhibition activity of both papaya peel and seed extracts were much lower. ABTS radical inhibition activity of papaya peel and seed extracts were ranged from the lowest $4.39 \pm 0.60\%$ and $1.28 \pm 0.24\%$ respectively to the highest $28.30 \pm 1.13\%$ and $11.19 \pm 0.62\%$, respectively. As compared to the papaya seed extract, papaya peel extract exhibited a significantly higher ($p < 0.05$) inhibition activity at the extract concentration of 200 and $500 \mu\text{g/mL}$. This is in agreement with Prasad et al. [19] that the peel of *Canarium odontophyllum* fruit exhibited higher inhibition activity than the seed extracts.

Lowest EC_{50} concentrations were exhibited by α -tocopherol and BHA, which were smaller than 0.001 mg/mL , followed by AA (0.081 mg/mL), papaya peel (5.47 mg/mL) and lastly papaya seed (9235.84 mg/mL) (Table I). The result strongly suggested that both papaya peel and seed extracts are weak ABTS radical cation inhibitors as compared to those natural and synthetic antioxidants.

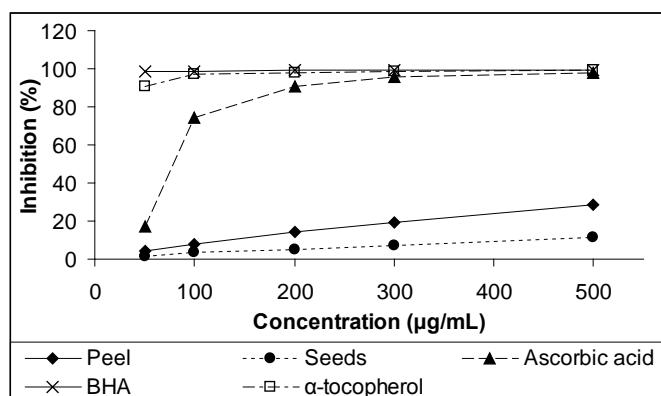


FIGURE 4 ABTS RADICAL CATION INHIBITION (%) OF PAPAYA PEEL AND SEEDS EXTRACTS. RESULTS ARE PRESENTED AS MEAN \pm STANDARD DEVIATION (N=3).

Conclusions

Papaya peel and seeds extracted using the optimised extraction conditions have exhibited antioxidant potential. Different concentrations of the extract studied have shown good linearity for the antioxidant capacity assays. Antioxidant capacities of papaya peel and seed extracts were much lower in comparison to the α -tocopherol, ascorbic acid, and BHA. The antioxidant potential of papaya peel and seeds may contribute to production of functional foods and nutraceutical in the near future utilising these papaya wastes.

ACKNOWLEDGEMENT

The financial and laboratory support from the Faculty of Applied Sciences, UCSI University is highly acknowledged.

AUTHORS DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

- [1] Moure, A., Cruz, J.M., Franco, D., Domínguez, J.M., Sineiro, J., Domínguez, H., Núñez, J., and Parajó, J.C., "Natural antioxidants from residual sources," *Food Chem.*, vol. 72, pp. 145–171, 2001.
- [2] Okonogi, S., Duangrat, C., Anuchpreeda, S., Tachakittirungrod, S., and Chowwanapoonpohn, S., "Comparison of antioxidant capacities and cytotoxicities of certain fruit peel," *Food Chem.*, vol. 103, pp. 839–846, 2007.
- [3] Peschel, W., Sánchez-Rabaneda, F., Diekmann, W., Plescher, A., Gartzia, I., Jiménez, D., Lamuela-Raventós, R., Buxaderas, S., and Codina, C., "An industrial approach in the search of natural antioxidants from vegetable and fruit wastes," *Food Chem.*, vol. 97, pp. 137–150, 2006.
- [4] Sancho, L.E.G.G., Yahia, E.M., and González-Aguilar, G.A., "Identification and quantification of phenols, carotenoids, and vitamin C from papaya (*Carica papaya* L., cv. Maradol) fruit determined by HPLC-DAD-MS/MS-ESI," *Food Res. Int.*, vol. 44, pp. 1284–1291, 2011.
- [5] Adebisi, A., Adaikan, P.G., and Prasad, R.N.V., "Tocolytic and toxic activity of papaya seed extract on isolated rat uterus," *Life Sci.*, vol. 74, pp. 581–592, 2003.
- [6] Thomás, G.E., Rodolfo, H.G., Juan, M.D., Georgina, S.F., Luis, C.G., Ingrid, R.B., and Santiago, G.T., "Proteolytic activity in enzymatic extracts from *Carica papaya* L. cv. Maradol harvest by-products," *Process Biochem.*, vol. 44, pp. 77–82, 2009.
- [7] Ng, L.Y., Ang, Y.K., Khoo, H.E., and Yim, H.S., "Influence of different extraction parameters on antioxidant properties of *Carica papaya* peel and seed," *Res. J. Phytochem.*, vol. 6, pp. 61–74, 2012.
- [8] Dubost, N.J., Ou, B., and Beelman, R.B., "Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity," *Food Chem.*, vol. 105, pp. 727–735, 2007.
- [9] Ferreira, I.C.F.R., Baptista, P., Vilas-Boas, M., and Barros, L., "Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal; Individual cap and stipe activity," *Food Chem.*, vol. 100, pp. 1511–1516, 2007.
- [10] Tsai, S.Y., Huang, S.J., Lo, S.H., Wu, T.P., Lian, P.Y., and Mau, J.L., "Flavour components and antioxidant properties of several cultivated mushrooms," *Food Chem.*, vol. 113, pp. 578–584, 2009.
- [11] Biglari, F., AlKarkhi, A.F.M., and Easa, A.M., "Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran," *Food Chem.*, vol. 107, pp. 1636–1641, 2008.
- [12] Cheung, P.C.K., and Lo, K.M., "Antioxidants activity of extracts from the fruiting bodies of *Agrocybe aegerita* var. Alba," *Food Chem.*, vol. 89, pp. 533–539, 2005.

- [13] Ismail, H.I., Chan, K.W., Mariod, A.A., and Ismail, M., "Phenolic content and antioxidant activity of cantaloupe (*Cucumis melo*) methanolic extracts," *Food Chem.*, vol. 119, pp. 643–647, 2010.
- [14] Ahmadi, F., Kadivar, M., and Shahedi, M., "Antioxidant activity of *Kelussia odoratissima* Mozaff. in model and food systems," *Food Chem.*, vol. 105, pp. 57–64, 2007.
- [15] Akond, A.S.M.G.M., Khandaker, L., Hossain, K.G., and Furutu, Y., Y., "Total polyphenol, polyphenol oxidase, antioxidant activity and color profiles of some wheat varieties from Bangladesh," *J. Agric. Biol. Sci.*, vol. 6, pp. 186–190, 2010.
- [16] Prior, R.L., Wu, X., and Schaich, K., "Standardized methods for the determination of antioxidants capacity and phenolics in food and dietary supplements," *J. Agric. Food Chem.*, vol. 43, pp. 401–403, 2005.
- [17] Tyug, T.S., Prasad, K.N., and Ismail, A., "Antioxidant capacity, phenolics and isoflavones in soybean by-products," *Food Chem.*, vol. 123, pp. 583–589, 2010.
- [18] Özyürek, M., Bektaşoğlu, B., Güçlü, K., Güngör, N., and Apak, R., "Simultaneous total antioxidant capacity assay of lipophilic and hydrophilic antioxidants in the same acetone-water solution containing 2% methyl- β -cyclodextrin using cuprin reducing antioxidant capacity (CUPRAC) method," *Anal. Chim. Acta*, vol. 630, pp. 28–39, 2008.
- [19] Prasad, K.N., Chew, L.Y., Khoo, H.E., Yang, B., Azlan, A., and Ismail, A., "Carotenoids and antioxidant capacities from *Canarium odontophyllum* Miq. fruit," *Food Chem.*, vol. 124, pp. 1549–1555, 2010.

Yee Kwang Ang earned his B.Sc. (Hons) Nutrition and Community Health from Universiti Putra Malaysia, Serdang,

Selangor, Malaysia. After three years of working in the food industry, he pursued his interest in M.Sc. majoring in Community Nutrition under the Universiti Putra Malaysia Graduate Research Fellowship.

Currently, he is a lecturer in the Faculty of Applied Sciences, UCSI University, Kuala Lumpur. His research focus is in the area of nutritional assessment, cancer and health education and promotion, and food science.

Mr Ang is a life-member of the Golden Key International Honour Society and the Nutrition Society of Malaysia. He is also a volunteer member of the National Cancer Society of Malaysia.

Winne Chiaw Mei Sia obtained her B.Sc. (Hons) Food Science & Nutrition from UCSI University, Kuala Lumpur, Malaysia. She works as a tutor with the Faculty of Applied Sciences, UCSI University, and at the same time pursuing her M.Sc. Nutritional Sciences at Universiti Putra Malaysia. Her current research interests include screening and identification of antioxidative compounds from local fruits and vegetables' by-products.

Hock Eng Khoo graduated from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM), and has obtained his B.Sc. (Hons) and M.Sc. degrees in Nutritional Sciences. He was sponsored by Ministry of Higher Education Malaysia as a MyBrain scholar for his Ph.D. degree at UPM. His research interest is on bioactive compounds in fruits and vegetables. He has been working on antioxidant research that focusing on carotenoids and polyphenols. He is one of the authors for over thirty local and international journal articles, conference proceedings and book chapters.

Hip Seng Yim graduated with a B.Sc. (Hons) Nutrition & Community Health and a M.Sc. Community Nutrition from Universiti Putra Malaysia, and he pursued his Ph.D. Food Science at Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia. He is currently a senior lecturer with the Faculty of Applied Sciences, UCSI University, and Kuala Lumpur. His current research interests include screening and identification of antioxidative compounds from wild edible mushrooms, and from local fruits and vegetables' by-products. He is a life-member of the Nutrition Society of Malaysia.